

Assessment of a Siloxane Poly(urethane-urea) Elastomer Designed for Implantable Heart Valve Leaflets

Chris Jenney, Peter Millson,* David W. Grainger, Robert Grubbs, Pathiraja Gunatillake, Simon J. McCarthy, James Runt, and Jason Beith


Synthetic polymer leaflets in prosthetic cardiac valves hold the potential to reduce calcification and thrombus, while improving blood flow, durability, and device economics. A recently developed siloxane poly(urethane-urea) (LifePolymer, LP) exhibits properties essential for heart valve leaflets, including low dynamic modulus, high tensile strength, minimal creep, and excellent biostability. LP's properties result from carefully designed "linked co-macrodial" chemistry that maximizes silicone content and virtual crosslinks between soft and hard phases. Characterization of multiple commercial batches demonstrates a robust synthesis process with minimal variation. Extensive ISO 10993-based biocompatibility testing results in no observable toxicity or other adverse reactions. An ex vivo AV shunt thrombogenicity investigation reveals nearly undetectable levels of platelet attachment and thrombus formation on LP surfaces. Chronic ovine implantation of prototype heart valves with LP leaflets shows no differences in thrombogenicity or systemic tissue response when compared to a clinically standard tissue-based valve. Toxicological risk assessment, based on extractables and leachables analysis of LP-based heart valves, confirms minimal toxicological risk. Lastly, 24-week, strain-accelerated in vivo LP biostability testing confirms previous favorable in vitro biostability findings. These studies demonstrate that this newly developed elastomer exhibits ideal biomaterial properties for the flexible leaflets of a totally synthetic heart valve replacement.

1. Introduction

As (AB)_n-type segmented block copolymers, polyurethanes (PUs) are comprised of three primary building block chemistries in tailored covalently linked sequences to yield polymer chain segments. These PU building block chemistries generally are 1) macrodiol or polyol, 2) diisocyanate, and 3) chain extender. Myriad chemical combinations of these three components, each with several different chemistry possibilities, result in a substantial array of PUs with interesting ranges of useful properties.^[1,2] Tailored PU mechanical and chemical properties derive from their molecular-level construction that yields distinct hard segments and soft segments which self-organize based on intermolecular forces (e.g., chain hydrogen bonding), segment association, and relative immiscibility to create microphase-separated polymer solids. PU microphase separation unique to each selected segment chemistry has long been proposed as essential to their compelling mechanical (e.g., tensile strength, elastic properties, hardness) and chemical behaviors (e.g., gas/water permeability, hydrolytic stability, biocompatibility).^[1–3]

Dr. C. Jenney, P. Millson, Dr. J. Beith
Research and Product Development
Foldax, Inc.
Salt Lake City, UT 84103, USA
E-mail: peter.millson@foldax.com

Prof. D. W. Grainger
Department of Biomedical Engineering
Department of Pharmaceuticals and Pharmaceutical Chemistry
University of Utah
Salt Lake City, UT 84112, USA

 The ORCID identification number(s) for the author(s) of this article can be found under <https://doi.org/10.1002/anbr.202000032>.

© 2020 The Authors. Advanced NanoBiomed Research published by Wiley-VCH GmbH. This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

DOI: 10.1002/anbr.202000032

Prof. R. Grubbs
Division of Chemistry and Chemical Engineering
California Institute of Technology
Pasadena, CA 91125, USA

P. Gunatillake
Commonwealth Scientific and Industrial Research Organization
Manufacturing
Clayton, VIC 3168, Australia

Dr. S. J. McCarthy
Portland, OR 97201, USA

Prof. J. Runt
Department of Materials Science and Engineering
Penn State University
University Park, PA 16802, USA

Biomedical PUs enjoy a substantial history of materials development and track records of success across a diverse palette of medical products.^[4] Numerous academic and clinical medical implant development experiences have demonstrated how PU chemistry is related to both its biocompatibility and biostability. Adoption and repurposing of early PU materials from other commercial applications for medical device use resulted in many successes and a small number of notable performance challenges. Specifically, commercialized polyetherurethane-based pacemaker lead insulation coatings and breast implant porous coatings both exhibited device-associated failures attributed to polymer degradation mechanisms catalyzed under physiological conditions.^[5] Oxidative degradation of the polyether soft segment associated with PU elastomeric properties was considered deleterious to these early implanted PUs.^[6,7] This link between PU segment chemistry and resulting in vivo PU implant performance served to distinguish the unique in vivo environmental demands for biomedical PUs, and motivated extensive and continuing efforts to improve PU biostability for long-term implantation.^[4]

One approach to reducing polyether-based PU oxidative degradation susceptibility has exploited new polyether polyol building blocks with fewer ether groups, such as poly(hexamethylene oxide) (PHMO), which has been shown to improve in vivo stability and, importantly, serve also to improve chemical compatibility of apolar siloxane (e.g., polydimethylsiloxane, PDMS, oligomers) soft segment building blocks.^[4,8–12] Substantial recent developments, including intelligent selection of PDMS terminal functional groups and the use of a second macrodiol as a segment compatibilizer, have enabled controlled phase mixing between PU soft and hard segments with superior mechanical properties and enhanced PDMS-PU in vitro hydrolytic resistance and in vivo biostability.^[4,13–16] Latest generation commercially available PDMS-based biomedical PUs utilize a two-step bulk polymerization process, allowing scalable mass production without metal catalysts and improved mechanical characteristics and biostability suitable for medical devices (e.g., Elast-Eon 2A, or E2A, AorTech Biomaterials, UK).^[17,18] E2A's superior biostability versus conventional polyether PU Pellethane 2363-80A has been shown in numerous ovine subcutaneous implantation studies.^[16,19–22] A translational success story, Abbott's E2A-based Optim cardiac lead insulation, has been clinically implanted since 2006, with over 6 million patients benefiting worldwide.^[23]

Despite their clinical success, recent in vitro studies using Arrhenius-accelerated hydrolysis have raised concerns over long-term siloxane-based PU biostability.^[24–27] A recognized primary limitation of Arrhenius-type, temperature-accelerated, in vitro degradation models is the high and sustained testing temperatures producing PU microstructural and morphological changes not observed in vivo.^[28] Most significantly, temperature-accelerated in vitro PU degradation results do not correlate with the very minimal changes seen in 5-year and 8 year clinical explants of Optim (E2A) pacemaker leads.^[29,30] An actively monitored registry study of the first commercially released Optim cardiac leads, encompassing 14 500 devices over 73 000 patient years, confirms Optim's in vivo stability with an outstanding all-cause device survival rate of 98.3% after more than 12 years of implantation.^[23] Given significant ambiguities in

accelerated in vitro testing to predict actual PU in vivo stability, future focus should be on improving these in vitro models and validation against long-term implant studies. Establishing strong in vitro–in vivo correlations is required to confidently accelerate further development of siloxane-based PUs central to several important medical device classes in clinical use, including prosthetic heart valves.

Prosthetic heart valves are well-established, lifesaving medical devices. Although complications such as thrombosis and calcification do occur, the rates are relatively low and valve performance and longevity are considered acceptable. The majority of heart valves implanted today feature leaflets made with fixed exogenous cardiac tissue, i.e., bovine pericardium or porcine valve leaflet. A smaller fraction of implanted heart valves utilize a rigid mechanical design featuring one or more pivoting carbon discs/leaflets. Carbon valves are acknowledged to be extremely durable; however, due to the nonphysiological flow and material surface chemistry, strong anticoagulation therapy is required and hemolysis is a concern. Tissue valves require only mild anticoagulation therapy, demonstrate good longevity, and can be delivered by a minimally invasive catheter; however, calcification complications remain a concern.

Because polymer properties can be carefully tuned and controlled, the possibility of using polymeric leaflets in prosthetic heart valves has been carefully explored by multiple organizations. In addition to physical properties, the geometry of polymer valve leaflets can be intentionally designed to ensure ideal physiological function, approaching that of native leaflets. Therefore, molded or solution-processed polymer heart leaflets offer the potential for low-cost, highly controlled, physiologic, minimally invasive prosthetic valves with excellent hemodynamics. Historically, many polymers have been explored as leaflet materials, including silicone rubber, polytetrafluoroethylene (PTFE), expanded PTFE (ePTFE), polyvinylalcohol (PVA), poly(styrene-isobutylene-styrene) (SIBS), and an array of polyurethanes.^[31] However, to date, no prosthetic valve with polymeric leaflets has achieved commercial success. Familiar clinical issues such as hemodynamics, thrombosis, and calcification, as well as in vivo degradation have been the primary challenges.

New PDMS-PU chemistries continue to be reported, seeking to improve in vivo biomaterials' performance using PU structure–property relationships.^[4] Arguably, siloxane-containing PUs exhibit the best biostability and biocompatibility to date, but frequently lack state-of-the-art mechanical properties. Application-specific properties for medical devices require control of specific PU mechanical properties together with robust, reliable biostability under implant conditions. Synthetic heart valve leaflets, for example, require high tensile and tear strengths, high resistance to creep deformation, and specific dynamic moduli. PUs were not capable of delivering these requisite properties until siloxane-based PUs with controlled microphase morphologies using new molecular and segmental architectures were produced, surpassing known mechanical properties for E2A.^[32–35]

This study reports chemical, in vitro, and in vivo analysis of a first-in-class biomedical-grade siloxane-based poly(urethane-urea) (SiPUU) (LifePolymer, LP, Foldax, USA) specifically developed for synthetic leaflets in a prosthetic heart valve (Tria, Foldax, USA). After significant development efforts, which

were thoroughly described in a series of publications, LP met property goals established to ensure proper and safe mechanical function of Tria valve leaflets, including ≥ 30 MPa ultimate tensile strength (UTS), $\geq 500\%$ elongation, ≥ 50 N mm⁻¹ tear strength, ≥ 200 J m⁻² tear energy threshold, 25–35 MPa dynamic modulus, creep rate < E2A, and biostability \geq E2A.^[32,35,36] LP's published synthesis and chemical design exploits five distinct prepolymer building blocks, including a diamine chain extender and siloxane-containing macrodiol and hard segment components, facilitating co-macrodiol soft segment interactions with hard segments using both carbamate and urea chemistry.^[32–35] LP soft segments are based on a 20:80 (w/w) mixture of 4,4'-methylenediphenyl diisocyanate-linked PHMO and telechelic α,ω -bis(6-hydroxyethoxypropyl)-PDMS. LP hard segments represent nearly 45% (w/w) of the total mass, comprising MDI and a mixture of 1,2-ethylenediamine (EDA) and 1,3-bis(4-hydroxybutyl)-1,1,3,3-tetramethyldisiloxane (BHTD).^[32] The creative urethane–urea polymer construction distinguishes this siloxane PU from E2A and another analogous commercial biomedical siloxane-based PU, PurSil35 (DSM Biomedical, NL), which each contain four linking components, but, notably, lack BHTD and the urea-linking chemistry that results from the inclusion of EDA. Innovative multistep, scalable polymer processing yields $\approx 50\%$ PU siloxane content, a silicone-rich PU surface, and high levels of hydrogen-bonded intersegment interactions between soft and hard segment phases.^[32,34] LP's molecular design is responsible for the demonstrated superior mechanical features and biostability necessary for use as a synthetic prosthetic heart valve leaflet.^[32–35] Building on the previously published exploration and characterization data, this study provides a wide range of additional in vitro and in vivo evidence supporting LP in this promising cardiovascular device application. These data include further material characterization, batch-to-batch consistency, ISO10993 biocompatibility, ex vivo thrombogenicity, extractables and leachables toxicological assessment, chronic ovine valve implants, and accelerated in vivo biostability.

2. Results

2.1. Characterization of Production-Scale SiPUU

An initial 14 LP synthetic batch has been completed commercially. Each was thoroughly characterized to ensure a consistent, high-quality polymer with specifications necessary for valve leaflet production. Data from these batches are shown in **Table 1** and examples of the raw data are included in the Supporting Information. Although many specifications remain proprietary, the interbatch production data demonstrate robust consistency. Standard deviations of the polymer mechanical properties are all within 6% of the averages and T_g standard deviations are < 3 °C. Even polymer molecular weight gel permeation chromatography (GPC) data, typically acknowledged to produce method-based variability of $\pm 10\%$, is within 16% of the mean value. Notably, molecular weight, ultimate tensile strength, and elongation averages are significantly above those reported for earlier SiPUU lab-scale development, while polydispersity index (PDI) remains consistent, indicating an improvement in process capability at the larger scale.^[32] The dynamic modulus, a critical

Table 1. SiPUU characterization data compiled from 14 commercial batches of LP.

Property	Analytical tool	Unit	Average	Std. Dev.
M_w	GPC	Da	552 800	87 100
M_n	GPC	Da	175 600	21 300
PDI	GPC	N/A	3.15	0.31
T_g (initial heat)	DSC	°C	−4.0	1.7
T_g (cooling)	DSC	°C	−7.8	2.5
T_g (reheat)	DSC	°C	−3.6	2.0
IR spectrum correlation to reference	ATR-FTIR	n/a	0.9977	0.0005
UTS	Tensile	MPa	38.3	1.8
Elongation	Tensile	%	760	43
Dynamic modulus	Dynamic tester	MPa	28.7	0.7
Tear energy threshold	Dynamic tester	J m ⁻²	291	73

parameter to ensure proper leaflet movement, blood flow, and overall valve sealing, is remarkably consistent and well within the 25–35 MPa target range identified during material development and valve leaflet specifications. The tear energy threshold, defined as the energy level that can be cyclically applied to a leaflet without propagation of a cut/tear, easily exceeds the minimum design requirement of 200 J m⁻² and provides $a > 2\times$ margin of safety during aortic valve function.^[37]

2.2. Aortic Valve Fabrication

The Foldax Tria Aortic Valve design (**Figure 1**) is a 100% synthetic polymer prosthetic aortic heart valve repeatedly fabricated for preclinical and biocompatibility testing. The valves consist of LP leaflets solution-cast onto a radio-visible polyether-etherketone (PEEK) stent and a PTFE felt sewing ring secured with polyester suture. Sterilization was achieved by standard ethylene oxide methods. The Tria valves fabricated for this study underwent all manufacturing processes/controls and met all visual, mechanical, and dimensional specifications necessary for human use.

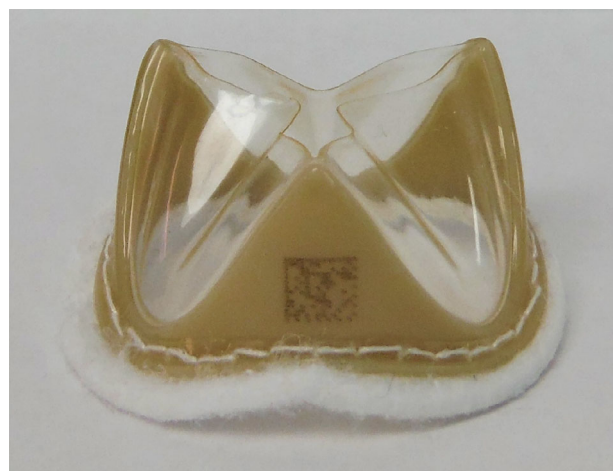


Figure 1. Foldax Tria Aortic Valve with LP leaflets, 23 mm outer diameter.

2.3. Biocompatibility

Because a complete medical device best represents patient exposure, the biocompatibility testing described in this study was performed on a complete, sterilized Tria heart valve with an approximate composition of 40 wt% LP, 40 wt% PEEK, and 20 wt% PTFE. Biocompatibility assessments were completed in compliance with ISO 10993 standards and Good Laboratory Practice (GLP) regulations. A summary of each test result is provided in **Table 2**. All biocompatibility testing met the relevant criteria, identifying no significant risk of toxicity or tissue injury. Successful ISO 10993 biocompatibility results, such as those detailed for the Tria valve with LP leaflets, are necessary to ensure

patient safety and secure regulatory approval of implantable medical devices worldwide.

2.4. Toxicological Risk Assessment from Exhaustive Extractables and Leachables Analysis of Tria Valves

Gas chromatography mass spectroscopy (GC-MS) and liquid chromatography mass spectroscopy (LC-MS) analysis of exhaustive Tria valve extracts (polar, semipolar, apolar) identified low levels of 37 unique compounds and 24 groups of related compounds (**Table 3**). Identical analysis of PBS and ethanol/water simulated-use leachables solutions identified low levels of 33 unique compounds and 11 groups of related compounds

Table 2. ISO 10993 biocompatibility testing results on Tria valves with LP leaflets.

Test description	Relevant Std	Results
Cytotoxicity – minimal essential medium elution assay	ISO 10993-5	Pass, noncytotoxic. The positive and negative control results met the validity criteria. At 24, 48, and 72 h, no reactivity, cell lysis, or reduction of cell growth was observed in the triplicate test article wells. Therefore, the test article received a grade of “0” and was considered noncytotoxic.
Intracutaneous reactivity test	ISO 10993-10	Pass, nonirritant. All assay validity criteria were met. Erythema and edema were not observed at any site on any subject at any time point (24, 48, or 72 h) for both normal saline extract and sesame seed oil extract. Therefore, the test article was considered a nonirritant.
Acute systemic toxicity test	ISO 10993-11	Pass, no acute systemic toxicity. All 20 test and control animals remained in overall good health over the course of the 72 h study. None of the animals treated with the test article extracts showed a significantly greater reaction than the animals treated with the negative controls. The test article showed no evidence of acute systemic toxicity.
Sensitization—guinea pig maximization	ISO 10993-10	Pass, nonsensitizer. All 36 test and control animals remained in overall good health over the course of the study. The overall pattern, intensity, and duration of reactions in the saline and sesame seed oil test article groups were identical to the negative control group, no erythema or edema observed. Based on the results of this study, the test article showed no evidence of delayed dermal contact sensitization and was considered a nonsensitizer.
Pyrogenicity—material mediated	ISO 10993-11	Pass, nonpyrogen. None of the three rabbits in this study showed a rise in body temperature $>0.1^{\circ}\text{C}$ above its control temperature. Therefore, the test article showed no evidence of material-mediated pyrogenicity.
Hemolysis assay, direct and extract methods	ISO 10993-4, ASTM F7756-13	Pass, nonhemolytic. Validity criteria for the positive and negative controls were met. The test article and test article extracts were determined to produce minimal hemolysis, equal to the negative controls. Therefore, the test articles were determined to be nonhemolytic using both direct and extract methods.
Partial thromboplastin time	ISO 10993-4, ASTM F2382	Pass, test article performed better than the reference material (HDPE). The replicate variability of the controls, as well as the reference material, met the criteria for a valid assay. The test article had an average clotting time of 110.1 s, which was 112% of the negative control, and was found to be statistically significant ($p < 0.05$). Therefore, the statistical analysis indicates the test article performed better than the reference material.
Complement activation	ISO 10993-4	Pass, test article complement activation is lower than the negative reference material (HDPE). Using the SC5b-9 assay, the test article exhibited activation at 6394 ng mL^{-1} and was 0.1% of the normalized SC5b-9 concentration produced by the positive control, cobra venom factor (CVF). In comparison, the negative reference material (HDPE) exhibited 0.1% activation relative to the CVF. The test article results were statistically significantly ($p < 0.05$) lower than the negative reference material.
Genotoxicity—AMES	ISO 10993-3	Pass, nonmutagenic. Under the conditions of this study, the mean number of revertant colonies for the test article was less than twofold over the mean number of revertant colonies for the negative control; therefore, the test article was considered nonmutagenic.
Genotoxicity—mouse lymphoma	ISO 10993-3	Pass, nonmutagenic and nonclastogenic. The controls for the assay performed as required, qualifying both the assay and cell culture system as valid. The mutant frequencies and cloning efficiencies of preparations treated with the test article were within the limits defined for a negative response. Accordingly, the test article is considered to be nonmutagenic and nonclastogenic.

Table 3. Quantity of leachable and extractable compounds from Tria valves identified by GC-MS and LC-MS.

		Exhaustive extraction			Leachables	
GC-MS	Total	0	24	56	3	8
	Unique	0	11	12 ^{a)}	3	6
	Related groups	0	5	4	0	1
LC-MS	Total	13 ^{b)}	91	72	9	109
	Unique	3 ^{b)}	8	2	7	17
	Related groups	4	7	4	1	9

^{a)}Additional two compounds identified in hexane also identified in ethanol/water extract; ^{b)}Two of the three compounds were identified by UV detection, not MS detection.

(Table 3). Examples of compound groups include ethylene glycols, fatty alcohols, siloxane cyclics, siloxane oligomers, dimethylacetamide (DMAc)-related compounds, methylene diisocyanate (MDI)-related oligomers, and MDI-diol-siloxane-related oligomers. The identification of very low levels of extractable compounds is typical, even for implant-grade polymers, due to the high sensitivity of these analytical methods.

As an outcome of the comprehensive toxicological assessment, margin of safety (MOS) values were calculated for each of the unique compounds and related compound groups identified by GC-MS and LC-MS. MOS values ≥ 1 indicate a minimal and acceptable risk of toxicity when the device is used as intended. MOS values < 1 require additional consideration to make a final toxicity determination. Minimal toxicity (MOS ≥ 1) was confirmed for all compounds leachable under simulated-use conditions and those compounds identified with polar and semi-polar solvents under aggressive, exhaustive extraction conditions. Only under the nonphysiological conditions of an exhaustive hexane solvent extraction at 50 °C for 72 h were compounds identified with a MOS less than 1.0, ranging between 0.5 and 0.9.

Inductively coupled plasma mass spectrometry (ICP-MS) identified eight metals, Mg, Al, B, Na, Si, K, Ca, and Ba, found in quantities of at least 1 µg per device in exhaustive water extractions, PBS leachables analysis, and ethanol/water leachables analysis. The MOS of these elements was remarkably high, ranging from 79 to 501 400. As a result, no toxicity concerns were related to extractable or leachable elements.

All available data were then considered to assess overall toxicology risk for the Tria valve with LP leaflets. Acknowledging the successful completion of ISO10993 biocompatibility testing (including acute systemic toxicity and genotoxicity), a minimal toxicity assessment from the simulated-use leachables analysis, and the understanding that the MOS from exhaustive extractions significantly overestimates potential biological exposure, the Tria valve with its three LP leaflets was concluded to represent minimal toxicity risk.

2.5. Subchronic Preclinical Histological Evaluation of Implant Tria Valves with LP Leaflets in Sheep

Eight (8) sterilized Tria valves (23 mm) with LP leaflets were successfully implanted in the aorta of eight sheep for a

140 day period. Human Carpentier-Edwards Perimount aortic valves (model 2900-25mm) were implanted in two sheep as controls.

No significant health problems were observed for the 10 valve-implanted sheep during the course of the 140 day study. Clinical chemistry data identified no significant trends. All animals gained weight throughout the study. No mineralization, hemorrhage, or valve surface thrombus was observed on either LP or control valves. In addition, both valve types demonstrated only minimal inflammation adjacent to the sutures and the aorta were found to be patent with no thrombi. The LP leaflets supported no fibrous tissue (pannus) or endothelialization. The control leaflets demonstrated a mild amount of fibrous tissue (pannus) coverage, but no endothelialization was observed. Overall, the assessed gross and microscopic histopathological parameters (Figure 2) of the Tria valve and adjacent tissues were similar or superior for the LP test articles compared to the control articles, had minimal severity, and did not create any clinically significant findings or safety concerns in this animal model.

Histopathological assessment of organs and tissue not in close proximity to the valves (kidneys, lungs, liver, gallbladder, spleen, lymph nodes, and brain) did not identify any notable findings unique to the Tria valve.

2.6. Ex vivo Nonhuman Primate AV Shunt Thrombogenicity

The ex vivo, nonhuman primate (NHP) AV shunt method previously described by Harker and Hanson was successfully used to evaluate the thrombogenicity of LP grafts ($n = 6$) and ePTFE control grafts ($n = 5$) (Figure 3).^[38] Overall platelet deposition on LP grafts was minimal ($< 0.08 \times 10^9$ platelets) for the entire 60 min study period (Figure 4). This low platelet deposition is an order of magnitude below historical published results on ePTFE grafts with clopidogrel (20 mg kg⁻¹) and aspirin (10 mg kg⁻¹), which had $2.0 \pm 0.4 \times 10^9$ platelets after 60 min.^[39] A statistical comparison of LP and ePTFE graft platelet deposition confirmed that ePTFE grafts underwent significantly ($p < 0.05$ in *t*-test) higher platelet attachment at 30, 45, and 60 min.

Fibrin accumulation on four of six LP grafts was sufficiently low to be below the gamma counter detection limit. The average amount of fibrin present on the LP devices was 0.003 ± 0.007 mg, orders of magnitude lower than the average ePTFE device fibrin deposition of 0.844 ± 1.156 mg. A single ePTFE replicate exhibited $> 8\times$ the fibrin accumulation (2.897 mg) of the average of the other four (0.331 mg). No justification for this outlying data point could be identified. The resulting standard deviation of the ePTFE fibrin data with this outlier was high and statistically significant differences versus LP were not identified (*t*-test, $p = 0.179$). Removal of this outlier from the ePTFE dataset results in a statistically significant difference ($p = 0.027$) between the LP and ePTFE samples.

2.7. In vivo Biostability from Prestrained LP Films in Rabbit Subcutaneous Implants

Prestrained LP and control polyurethane (Pellethane 80A and Pellethane 55D) dumbbells were successfully implanted subcutaneously above the dorsal muscle of eight (8) rabbits

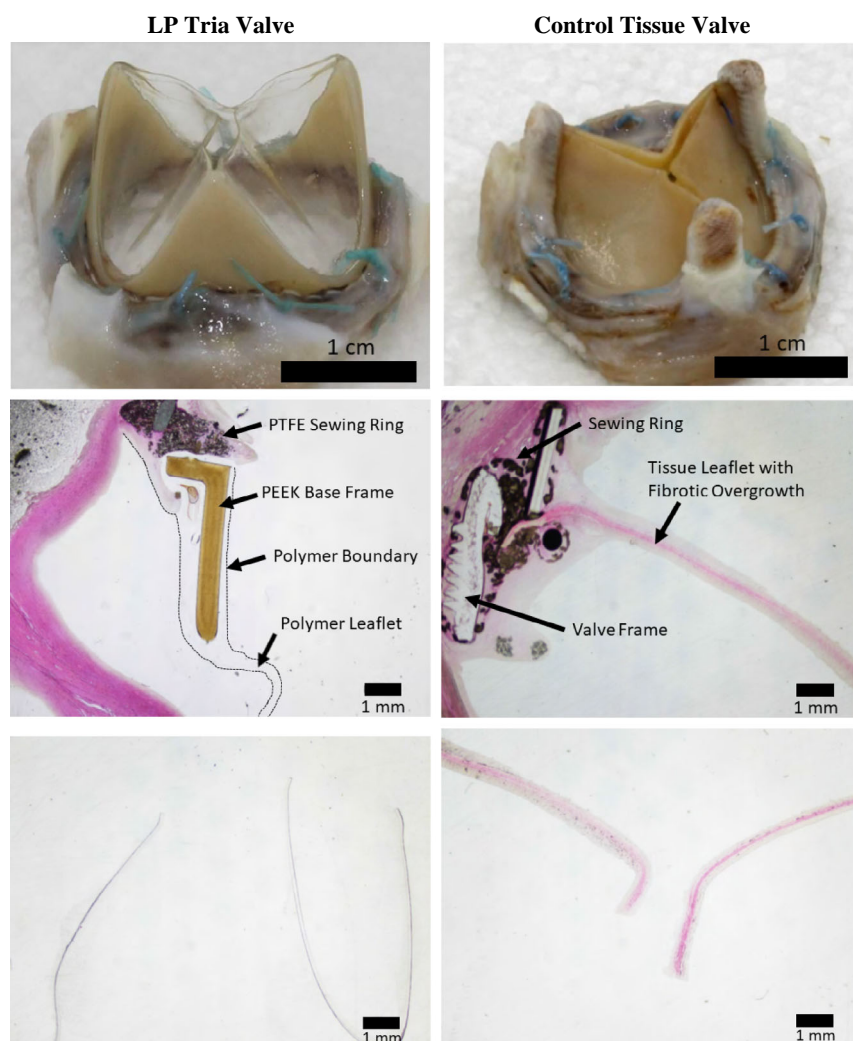


Figure 2. Representative 140 day ovine study explant pathology. Left: LP Tria valve images. Right: Carpentier-Edwards Perimount control tissue valve images. Top: gross images after removal of valves and trimming of aortic tissue. Middle: histopathology of frame, leaflet, and surrounding tissue. Bottom: histopathology of leaflet tips. All histopathology slides were prepared with H&E stain and are shown at a consistent magnification as demonstrated by the scale bars. Note that the LP material does not significantly absorb H&E stain; therefore, the LP leaflets are difficult to visualize. The boundary of the LP material has been highlighted with a dashed line in the middle-left image.

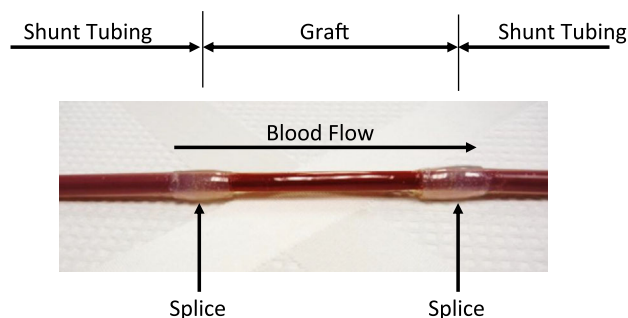


Figure 3. NHP blood circulating ex vivo through the LP graft (center) bonded to silicone rubber AV shunt tubing (left and right).

for 3–6 months. Sixteen (16) of each sample type were implanted. Of the 48 implanted samples, surgical complications or infection

required 10 samples (3 LP and 7 controls) to be explanted and removed from the study within 30 days of implant. No other implant-related health issues were noted during the study.

Figure 5 details the results of visual examination scoring, which attempts to classify the extent of any visible degradation. The 6 month rabbit model was successful in causing significant degradation (score of 6) in the positive control samples. A moderate amount of surface degradation was even identified in the negative controls (score of 4). Note that the Pellethane 55D Negative Control is a biomaterial successfully implanted clinically for decades, but has been shown to demonstrate mild degradation in vivo and in accelerated in vitro testing.^[30,40] Our control results confirm that the 150% strain level in this rabbit model is a strong accelerator of PU degradation. Even with this level of acceleration, the LP samples showed no visual signs of degradation (score of 1).

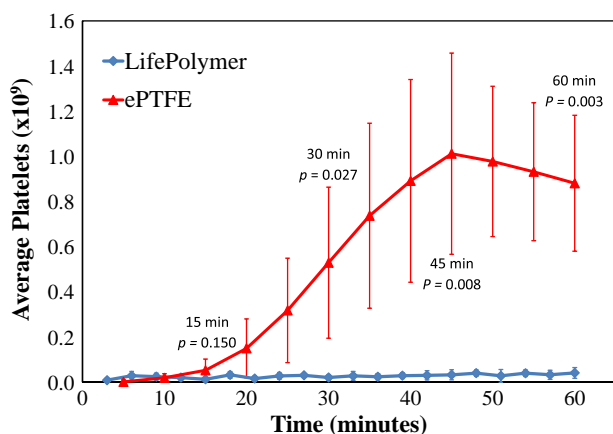


Figure 4. Platelet deposition on LifePolymer (LP) and ePTFE reference grafts in the NHP AV shunt loop. Data shown as average with error bars representing one standard deviation. *P*-values represent a two-sided *t*-test performed between the LP test group ($n = 6$) and the ePTFE reference group ($n = 5$) at specific time points.

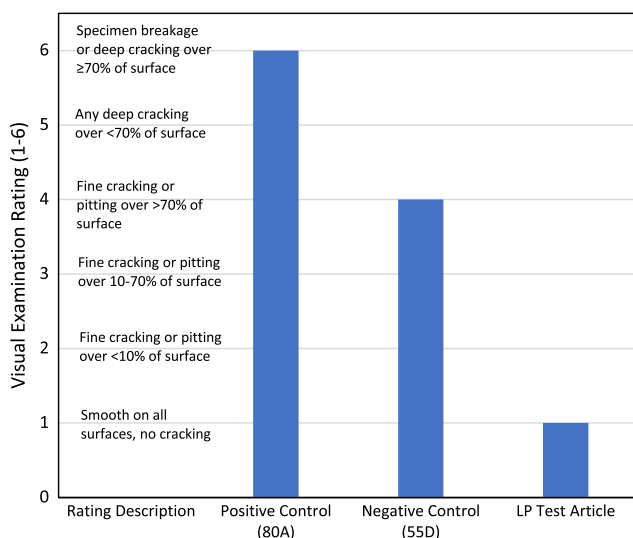


Figure 5. Assessment of degradation by visual examination of LP test article and PU control article cast films after 6 months in vivo under 150% strain. The definitions of each rating are provided on the left. The values shown in this graph represent an overall degradation assessment of each group.

Figure 6 provides typical field emission scanning electron microscope (FE-SEM) images and general observations for the LP and control groups after 6 months. Note that due to the challenges associated with assessing transparent sample surfaces with light microscopy, FE-SEM assessments may deviate somewhat from visual evaluation results of the same samples. The positive controls (Pellethane 80A) showed significant surface pits, cracking, and complete separation of the central region of one sample. The negative controls (Pellethane 55D) showed minor surface pitting and cracking at both time points. Note that the FE-SEM imaging identified less pitting and shallow cracking in the negative control than the visual examination.

The LP test articles showed no signs of pitting, cracking, or any other types of degradation even at 6 months.

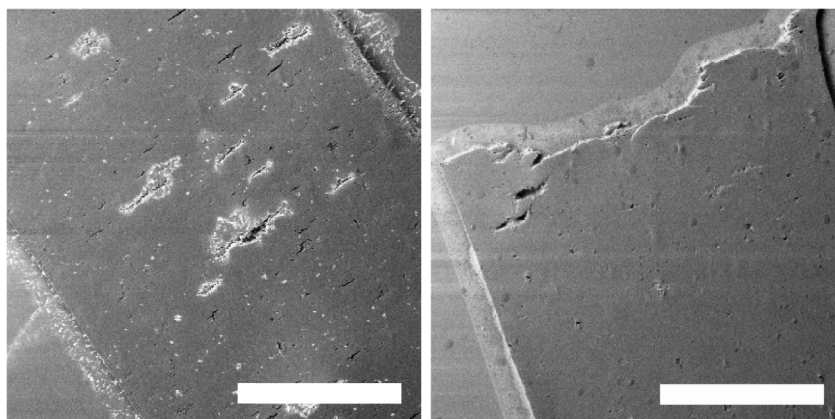
These study data show that the LP material is highly biostable, even in an in vivo model accelerated by strains that are $>10\times$ the expected strain in a valve leaflet. In addition, FE-SEM-energy dispersive X-ray analysis (EDAX) found no evidence of calcium deposition on the LP test article at either 3 or 6 month time points (data not shown).

3. Discussion

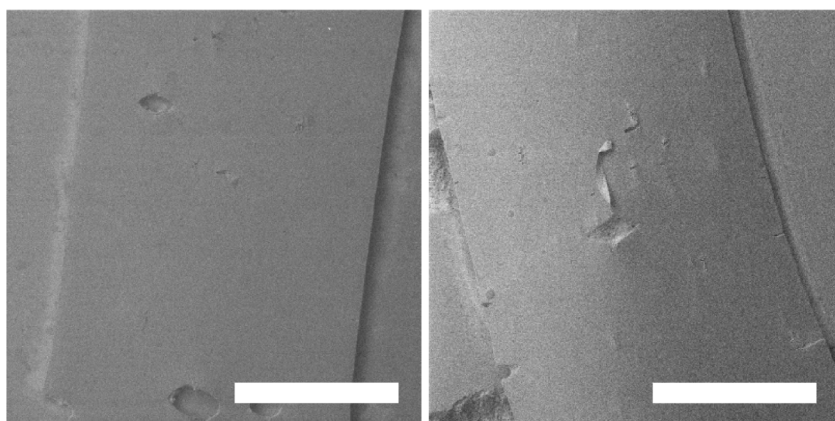
A completely synthetic and clinically reliable heart valve prosthesis has been elusive, requiring highly complex combinations of valve design, biomaterials combinations, manufacturing constraints, implant tools, and surgical techniques. The newly designed Tria aortic replacement valve contains elastomeric polymer leaflets of a new synthetic SiPUU commercialized as LifePolymer (LP, Foldax, USA). A series of previous publications has extensively described LP's synthetic chemistry, cast bulk film microstructure, silicon surface enrichment, unique polymer processing, and extensive physical properties relevant to synthetic heart valve application.^[32–35] Replacement of traditional xenogeneic pericardial tissue leaflets with high-performance SiPUU provides the advantages of a highly controlled leaflet design, ideal and tailored mechanical properties, a robust supply chain, and lower manufacturing costs. Nonetheless, translation, clinical testing, and commercialization of any replacement heart valve requires development and testing according to a number of guidance and standards documents, including ISO 10993 “Biological Evaluation of Medical Devices,” ISO 5840-1 “Cardiac Valve Prostheses: General Requirements,” and ISO 5840-2 “Cardiac Valve Prostheses: Surgically Implanted Heart Valve Substitutes.” Results from a series of studies intended to validate the biocompatibility, safety, and stability of the LP leaflet material and the Tria aortic valve prosthesis are aligned with these guidances. Together with the earlier reported work on this new SiPUU copolymer, an exhaustive data set now confirms LP's suitability for use as a leaflet in a replacement heart valve.^[32–35]

Many important chemical, thermal, and physical properties of LP made at the laboratory scale have been described during the LP biomaterial's early development.^[32–35] These include important benchmarks for LP's solvent-processable mechanical properties (elasticity, tensile strength, tear strength, creep) attributed to unique co-macrodial chain-extended segmental interactions with new hard segments also containing siloxane chemistry, cast surfaces consistently and substantially enriched in siloxane low-energy blocks, and both carbamate and urea linkages imparting stability and regular hydrogen bonding.^[33,34] Extending this bench synthesis to the first stages of device translation, multiple LP batches commercially synthesized at scale demonstrate a high level of process control. Modest enhancements in molecular weight, ultimate tensile strength, and elongation over previously reported lab-scale properties further indicate improvements in process capability at a larger scale.

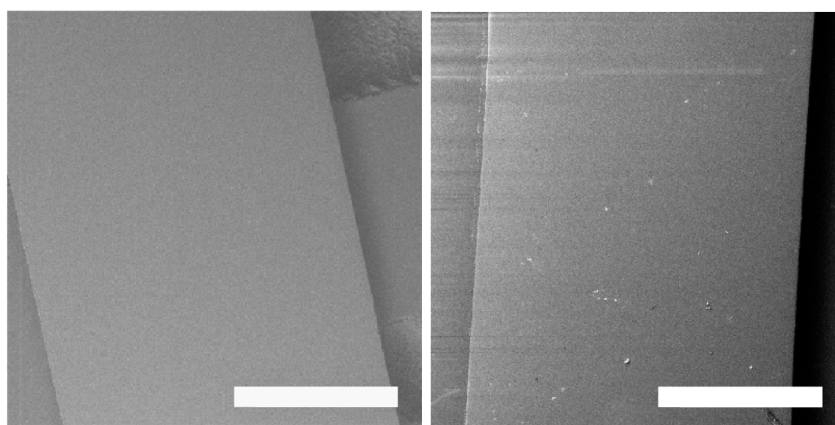
The ISO 10993 biological evaluation standards have been adopted globally as part of a risk management process for verifying the biocompatibility of finished medical devices, including



Positive Control (80A): Deep cracking, full thickness defects, and specimen breakage.



Negative Control (55D): Minor surface pitting and cracking was observed on all samples.



LP Test Material: Smooth on all surfaces. No cracking.

Figure 6. Representative FE-SEM images of LP test article and PU control article cast films after 6 months in vivo under 150% strain. Images show the center of two representative explanted dogbones from each group. Scale bars = 500 μ m.

heart valves. Because a heart valve remains in long-term blood and tissue contact, ISO 10993-1 requires the most rigorous battery of tests, including cytotoxicity, intracutaneous reactivity, material-mediated pyrogenicity, systemic toxicity, implantation effects, hemocompatibility, and genotoxicity. A carcinogenicity

assessment is also expected if the device contains any known mutagens, carcinogens, or reproductive toxins.

The Tria valve with LP leaflets passed all standard biocompatibility testing, identifying LP in this context as noncytotoxic, a nonirritant, a nonsensitizer, nonpyrogenic, nongenotoxic,

nonclastogenic, and producing no acute systemic toxicity (Table 2). Longer term subchronic toxicity testing in an ovine model identified no risk of localized or systemic toxicity. The Tria valve materials were also determined to be nongenotoxic by both standard AMES and mouse lymphoma methods. These results and the absence of any known genotoxic or carcinogenic compounds in the LP formulation justified not performing a carcinogenicity test, in agreement with ISO 10993-1 guidelines.

As per ISO 10993-4, the blood compatibility of any heart valve must be assessed with both hemolysis and in vivo/ex vivo thrombosis studies. As shown in Table 2, the Tria valve materials were nonhemolyzing and demonstrated acceptably low rates of coagulation with partial thromboplastin time and complement activation in vitro studies. The ex vivo thrombosis assay using the LP-based AV NHP shunt (Figure 4) further supported low coagulation activation by LP, producing nearly undetectable levels of platelet attachment and fibrin deposition in situ, both key markers of thrombosis. Essential for any heart valve leaflet material, the Tria valve and LP leaflets have both shown exceptional hemocompatibility in two different large animal models (see Figure 2 and 4). This is attributed to high, nonstoichiometric levels of siloxane at the LP leaflet surface, known to favor hemocompatibility.^[32]

A substantial fraction of adverse effects from implanted medical devices are attributed to the unintended release of compounds or elements.^[41] Polymeric biomaterials in medical devices may contain extractable and potentially toxic low-molecular-weight species, such as oligomers, plasticizers, antioxidants, and processing aids. For this reason, ISO 10993 biocompatibility testing uses extraction techniques to isolate these compounds for biological assessments. A typical complementary analytical approach described by ISO 10993-18 is the identification, quantification, and toxicological assessment of compounds and elements that are extractable under simulated-use conditions (leachables) and/or using aggressive extraction solvents and conditions, an “exhaustive extraction.” In this manner, the toxicological safety of a device can be confirmed not only with biological testing but by state-of-the-art chemical analysis, such as GC-MS, LC-MS, and ICP-MS, coupled with toxicological risk assessment.

It is important to recognize that the calculation of the MOS value is only one element of a toxicological assessment and it involves multiple highly conservative assumptions. First, the calculation assumes that 100% of each compound extracted would be bioavailable within 24 h, with the same quantity released daily over the lifetime of the patient. Under actual use conditions, compounds from a one-time implant are expected to leach out over weeks, months, or even years, at ever-decreasing concentrations, resulting in much lower toxicity risks. In addition, under actual use conditions, many compounds identified specifically by this harsh apolar solvent extraction may never be released from the valve in vivo. This can be seen when comparing the exhaustive extraction data with the leachables data. Thus, the MOS calculations significantly overestimate potential biological exposure and toxicological risk.

After considering the entirety of the data, including the nontoxic assessment of leachable compounds and elements, the toxicological data from the exhaustive extractions, and the

successful ISO 10993-1 biocompatibility test results (Table 2), including acute systemic toxicity, genotoxicity, and ex vivo thrombogenicity (Figure 4), the Tria valve with its three LP leaflets was concluded to represent minimal toxicity risk.

Implant biocompatibility and biological safety, particularly in the context of a device mechanical function, as for heart valve leaflets, generally includes further assessment of material biostability. ISO 10993 parts 13, 14, and 15 describe testing protocols designed to identify compounds produced as a result of biomaterial degradation. In vitro studies are appropriate, if guidance documents or literature reports provide a methodology relevant to the device and specific materials being considered. Previous publications demonstrate generally outstanding in vitro and in vivo resistance of LP to aggressive oxidative attack.^[34–35] Many publications over the past 40 years have used an accelerated in vivo model of polymer degradation for PU elastomers.^[20,42–43] The 6-month rabbit implant study described accelerated polymer degradation in vivo by applying a constant 150% strain to the material just prior to subcutaneous implantation. This level of strain is $>10\times$ the strain expected to be seen in an aortic valve leaflet. Consistent with previous studies, this level of applied strain elicits significant pitting, cracking, and material degradation in a soft polyetherurethane, such as Pellethane 80A (Figure 6). Even Pellethane 55D, with successful clinical use in a variety of implant applications, does not avoid degradation in this aggressive test (Figure 6). By contrast, the strained LP samples showed no signs of degradation (e.g., pitting or cracking) even after 6 months of implantation under these accelerated conditions. A similar level of biostability has been previously demonstrated by other siloxane–urethanes, including ElastEon2A and PurSil, both of which have successful implant applications.^[20,42] Addition of unique new urea segment bonds and a siloxane-containing BHTD chain extender to enhance LP’s mechanical properties required for effective valve leaflet function has not compromised its in vivo biostability in this accelerated assessment.

4. Conclusion

Mechanical and biological performance requirements for heart valve prosthesis leaflets are very demanding. Despite the indisputable advantages of a synthetic polymer leaflet, no examples capable of reliable device commercial translation are known. Substantial prior work highlights the focused development of a siloxane polyurethane-urea biomaterial intended solely for use as a heart valve leaflet.^[32–35] Incorporating a high level of siloxane and optimized levels of urea linker chemistry, a linked co-macrodial, and a silicone-containing hard segment, LP demonstrates an ideal dynamic modulus for leaflet use with a high mechanical strength, low creep, high tear energy threshold, and both in vitro and in vivo biostability. Scaled batch-to-batch polymer production consistency, excellent ISO 10993 biocompatibility and ex vivo thrombosis results, nonconcerning toxicological risk assessments, successful chronic sheep implantation, and promising rabbit biostability testing results all confirm the biological safety of the LP biomaterial in the Tria valve device design. These new and promising results, together with the attractive original LP property set from lab-scale production, provide a

diverse and substantial evidence base to proceed with the application of LP leaflets in a totally synthetic heart valve.

5. Experimental Section

Siloxane-Based Poly(urethane-urea) (SiPUU) Synthesis: As previously described, LP SiPUU was synthesized with a multistep synthetic process.^[32] Briefly, α,ω -bis(6-hydroxyethoxypropyl)poly(dimethylsiloxane) (PDMS) was purified in a wipe film evaporator to remove low-molecular-weight cyclic siloxane compounds. Linked PHMO was synthesized by reacting a 2:1 molar ratio of PHMO and MDI at 80 °C for 2 h. Prepolymer was prepared by blending together PDMS and linked PHMO, adding MDI titrated to ensure complete reaction of the polymer, and stirring for 2 h at 80 °C. Chain extension began with the addition of 1,3-bis(4-hydroxybutyl)-1,1,3,3-tetramethyldisiloxane (BHTD) and a 2 h reaction period. The final steps of the chain extension were the dissolution of 10% w/v DMAc followed by the addition of ethylene diamine (EDA). The chain extension was complete over 3 h. The resulting approximate molar composition of LP is 50% MDI, 3% linked PHMO, 17% PDMS, 15% BHTD, and 15% EDA. No processing aids, antioxidants, or other additives are present in LP. For testing purposes, LP films (200 μ m thickness) were solution-cast from each batch of LP, using LP–DMAc solutions (25% w/w) applied to glass plates using a film casting knife, then dried at 55 °C overnight.

The molecular weight (M_w , M_n , PDI) of each batch of LP–DMAc solution was determined with a GPC system consisting of an HPLC pump/controller, RI detector, Styragel HT2, HT3, HT4, HT5 columns (Waters) at 80 °C, and a 1.0 ml min^{−1} flow of mobile phase (DMAc with 50 mM LiBr). Molecular weight values were reported relative to monodisperse polystyrene standards.

Glass transition temperature (T_g) was determined with a DSC (Discovery, TA Instruments). Dry film samples (3–5 mg) from each batch were equilibrated at −90 °C, then taken through heating (10 °C min^{−1}), cooling (5 °C min^{−1}), and reheating (10 °C min^{−1}) cycles between −90 and 100 °C. T_g values during heating, cooling, and reheating were determined.

Films from each LP batch were spectroscopically analyzed using a diamond attenuated total reflectance Fourier transform infrared spectroscopy (ATR–FTIR) system (iS50, ThermoFisher) over a spectral range of 525–4000 cm^{−1}, averaging 32 scans at a resolution of 4 cm^{−1}. Each spectrum was compared to a historical reference spectrum using a correlation coefficient calculation (QCheck, Omnic software). A coefficient of 1.0 indicates a perfect match.

Seven (7) replicate ISO 37 type-2 dog bones were cut from a cast LP film from each batch. Using a tensile testing system (Mark-10), dog bones were pulled in tension to failure at 100 mm min^{−1} under ambient conditions. UTS and elongation to failure were calculated for each LP batch.

For each batch, six (6) replicate samples (10 mm × 42 mm) were punched from an LP film and a cut of controlled depth was made on one edge of each sample. Using a mechanical tester (Electroforce 3200, Bose Instruments), these samples were subjected to a set of 50 000 tensile fatigue cycles at 10 Hz and a maximum stress of 0.5 MPa in 37 °C water. Samples were then subjected to sets of 50 000 fatigue cycles at increasing stress levels in 37 °C water until the crack/tear growth exceeded 0.1 mm cycle^{−1}, at which point a tear energy threshold (J m^{−2}) was calculated based on the energy of loading and the sample dimensions.^[37] Dynamic modulus (MPa) was calculated as the slope between the minimum and maximum points on the stress–strain curve during cyclic loading at the 0.5 MPa stress level (Figure S5, Supporting Information).

Aortic Valve Fabrication: The Foldax Tria Aortic Valve design (Figure 1) is a 100% synthetic polymer prosthetic aortic heart valve with a flexible, radio-visible PEEK (Zeniva, Solvay) stent and annular base with diameters between 17 and 27 mm. Three flexible LP leaflets with specifically designed thickness profiles were fabricated in place, on the frame, using a carefully controlled solvent casting process. Dipping speed and orientation, LP polymer solution (in DMAc) viscosity, environmental temperature and humidity, and drying parameters were critical to ensuring that cast leaflets

met dimensional specifications with micron-level precision. After polymer film casting, a mechanical trimming step was performed to finalize LP leaflet dimensions. A PTFE felt sewing ring was secured onto the annular PEEK valve base using a polyester suture running through a series of regularly spaced holes in the base outer annulus. Sterilization was achieved by standard ethylene oxide methods.

Biocompatibility Assessments: A series of ISO 10993-based biocompatibility tests was performed on sterilized Tria Aortic Valves with LP leaflets. A complete Tria heart valve consists of LP leaflets (≈40% w/w), a rigid frame made of implant-grade PEEK (≈40% w/w), a sewing ring (≈20% w/w) made of implant-grade PTFE secured with a commercially available polyester suture. Table 2 provides an overview of the specific tests performed, all of which were performed in compliance with FDA GLP regulations (21 CFR Part 58). Summaries of the test methods are provided in Table S2, Supporting Information.

All preclinical biostability studies were performed in accordance with the US National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals, which includes approval by the test facility Institutional Animal Care and Use Committee (IACUC). The test facility was registered with the US Department of Agriculture (USDA, #41-R0074) and accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC, aaalac.org).

Toxicological Risk Assessment from Exhaustive Extraction and Leachables Analysis: Potential toxicological risks of the Foldax Tria Aortic Valve, including the LP leaflets, were assessed using exhaustive extraction performed in triplicate with water (polar), 50% ethanol/water (semipolar), and hexane (nonpolar) solvents, all at 50 °C for 72 h. Any compounds/elements extracted from the valves were identified by quadrupole time-of-flight gas chromatography mass spectrometry (QTOF–GCMS), quadrupole time-of-flight liquid chromatography mass spectrometry (QTOF–LCMS), and ICP–MS. Relative quantification was performed in triplicate by QTOF–GCMS and ultra high pressure liquid chromatography with charged aerosol detection and ultra violet detection. See the Supporting Information for additional analytical method details. Grouping of compounds was performed when consistent toxicological data were available. The total concentration per device for a compound group represented a sum of the amounts for each individual compound within a group. All compounds found with maximum levels above the analytical evaluation threshold (AET) of 1.5 μ g device^{−1}, a limit supported by the ICH M7 guidance, were reported. A reporting limit of 1.0 μ g device^{−1} was used for ICP–MS elemental data. Data from the exhaustive extractions were key inputs in the subsequent assessment of toxicological risk using methods consistent with ISO 10993-17:2002 R2008.

A biologically relevant, simulated-use leachables analysis was also performed in triplicate, using both PBS (polar) and 40% ethanol/water (semipolar) as extraction solvents at 37 °C for 72 h. Identification and quantification of compounds was performed in an identical manner as the exhaustive extraction described previously.

The toxicological evaluation/risk assessment process for both leachables and exhaustive extraction was performed by an experienced toxicologist and followed the general approach described in ISO 10993-17:2002 R2008. The identification and characterization of potential deleterious effects of exposure to extractable chemicals/elements included the establishment of a tolerable intake, TI [μ g kg^{−1} day^{−1}], for the chemicals/elements and calculation of the human dose equivalent, HDE [μ g kg^{−1}], and a margin of safety, MOS = TI/HDE. Due to the demonstrated nonmutagenic nature of the Tria heart valve, the default TI used for compounds without alternative data was 1.5 μ g kg^{−1} day^{−1}, the Cramer class III Threshold of Toxicological Concern (TTC).

Subchronic Toxicity (140 Day) Preclinical Evaluation: Tria aortic valves with LP leaflets were evaluated in the healthy ovine model over an implant period of 140 days. Sterilized Foldax Tria valves (23 mm) were implanted in eight animals (n = 8 test samples). A sterile human Carpentier–Edwards Perimount aortic bioprosthesis model 2900–25mm was implanted in two animals as a clinically acceptable, benchmark control device (n = 2 controls).

Animals were screened for compatible annulus size prior to enrollment. A total of 10 (2 female, 8 male) sheep, 40–60 kg in weight, underwent a left

thoracotomy and were prepared for cardiopulmonary bypass. Once on bypass, the aorta was opened and the test or control device was implanted as per the manufacturer's instructions. During the procedure, the activated clotting time was maintained above 250 s by the administration of intravenous heparin, as needed. The aorta was then sutured closed and the animals were weaned off bypass. No postop anticoagulant therapy was administered. General health and clinical chemistry (CBC, serum chemistry, LDH, haptoglobin, free hemoglobin, reticulocyte count, coagulation panel) were assessed regularly, beginning at day seven and monthly thereafter. At approximately 140 days postimplant, the animals underwent a sedated terminal procedure and a gross necropsy was performed. The valve, heart, and lungs, as well as tissues from the lymph nodes, liver, spleen, kidney, and brain, were collected for histological evaluation. All histopathological data collected from the Tria aortic valve were compared directly to the control valve.

This preclinical study was performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals, which includes approval by the test facility IACUC. The test facility was registered with the USDA (#41-R0074) and accredited by AAALAC (aaalac.org).

Ex Vivo AV Shunt Thrombogenicity Study: Six (6) LP grafts (4 mm diameter, 70 mm long) were prepared by dip coating a stainless steel mandrel in a 22% (w/w) LP/DMAc solution. Each sample was dip coated four times in a glove box at 85 % RH. Between each coating, samples were rotated in an oven at 55 °C for 30 min. After the final coating, samples were held in a 55 °C oven overnight. LP grafts were removed from the mandrels under running RO water. Grafts were packaged and sterilized by ethylene oxide.

Silicone rubber tubing (4.0 mm ID, 6.5 mm OD) was abutted to both ends of the grafts and bonded with silicone medical adhesive and an overlying 15 mm length of fluorinated ethylene propylene heat shrink tubing (Figure 3).

The ex vivo, NHP AV shunt method previously described by Harker and Hanson was used to evaluate vascular graft thrombogenicity.^[38] A single baboon with no history of anticoagulation or antiplatelet therapy was utilized for this study. Six LP grafts were tested over the course of two days. CBC values and platelet counts were confirmed to be normal prior to the start of each study. A silicone rubber tubing AV femoral shunt was surgically established. Autologous ¹¹¹In-labeled platelets and ¹²⁵I-labeled fibrinogen (pooled) were introduced into the subject's circulation. The graft segment was primed with isotonic saline and added into the shunt circuit. Blood flow through the circuit was controlled with a single distal clamp and continuously monitored to be $\approx 100 \text{ ml min}^{-1}$. Platelet accumulation in the central 20 mm of the graft was measured every 3 min for 1 h by scintigraphy using a gamma camera. After the 1 h exposure, the graft segment was removed from the shunt circuit and flushed with isotonic saline until all visible blood was removed. The silicone tubing on either side of the LP graft was then removed, and the LP graft was stored at 4 °C for 30 days, allowing ¹¹¹In decay and quantification of ¹²⁵I-labelled fibrin/fibrinogen deposits on the central 50 mm of each LP graft with a gamma camera.

Platelet and fibrin deposition on the LP grafts were compared to historical data from ePTFE grafts ($n = 6$) of similar size, using *t*-tests at 15, 30, 45, and 60 min. A *p*-value of 0.05 was considered statistically significant.

This preclinical study was performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals, which includes approval by the test facility IACUC. The test facility was registered with the USDA (#92-R-001) and accredited by AAALAC (aaalac.org).

In Vivo Biostability in a Rabbit Model: Pellethane 2363-80A (Lubrizol), Pellethane 2363-55D (Lubrizol), and LP polymer solutions with 25% (w/w) solids content in DMAc were applied to a glass plate using a film casting knife. DMAc was evaporated in a 55 °C oven overnight. LP films were peeled off the glass plates under running RO water. Final film thicknesses were determined to be 0.5–0.7 mm. Samples were defined as

Positive Control: Pellethane 80A PEU (in vivo degradation well documented)^[4,19]

Negative Control: Pellethane 55D PEU (strong history of clinical use, acceptable biostability)^[4,19]

Test Article: LP SiPUU

Dumbbells of 3 cm length with holes in each end were punched from the sheets. Polycarbonate stretching mandrels were machined from sheet material. Dumbbell samples and mandrels were sterilized by ethylene oxide.

Under aseptic conditions, all polymer samples were strained with forceps and mounted onto polycarbonate mandrels, placing the sample end holes over the ends of the mandrels (Figure 7). Mandrel dimensions were carefully controlled to result in a 150% strain condition in the central, reduced cross-section region of the dumbbell.

Strained samples were aseptically implanted subcutaneously above the dorsal muscle of rabbits. One of each sample type was implanted using random site selection on each side of the spine to eliminate position bias (Figure 7). A total of 8 rabbits and 16 of each sample type were implanted. Postsurgical health checks and wound site observations were performed daily.

Rabbits were euthanized after 12 weeks ($n = 4$) or 24 weeks ($n = 4$), when samples were carefully removed from the surrounding tissue. Samples were stored in normal saline until evaluation.

Previous reports indicate that visual and SEM surface assessments are the most sensitive method for detecting PU degradation in this type of study. Therefore, the surfaces of all explanted samples were first evaluated visually using the scoring scheme as defined by Martin et al.^[16,44] 1 = specimen smooth on all surfaces, no cracking observed; 2 = patches of cracking or pitting over less than 10% of surface area; 3 = patches of cracking or pitting over 10–70% of surface area; 4 = fine cracking or pitting over greater than 70% of surface area; 5 = any deep cracking covering less than 70% of surface area; 6 = deep cracking covering more than 70% of surface area and/or specimen breakage.

In addition, all explanted samples were evaluated using FE-SEM-EDAX (FEI Quantum 600) for evidence of degradation, such as pitting and cracking, and calcium deposition.

The in vivo portion of this biostability study was performed in Australia and approved by the Commonwealth Scientific and Industrial Research Organization (CSIRO) North Ryde Animal Ethics Committee in accordance with the Australian Code for the Care and Use of Animals for Scientific Purposes.

Statistical Analysis: Continuous variables are expressed as mean \pm standard deviation. The statistical analysis of ex vivo platelet adhesion and thrombus formation was performed between the test and control

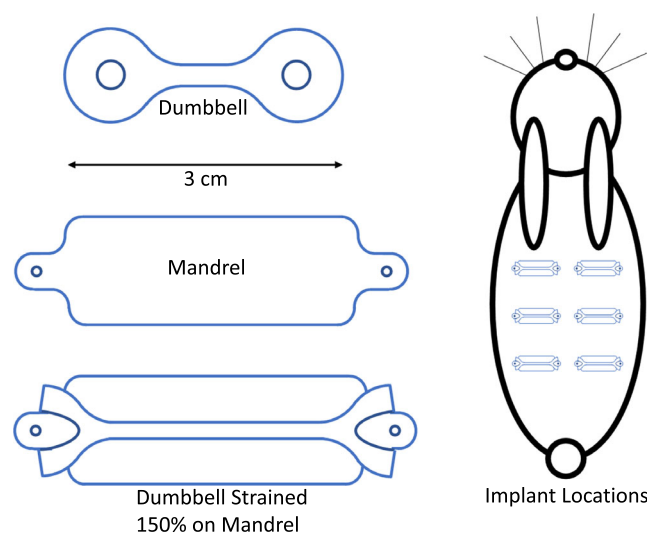


Figure 7. Rabbit biostability study sample construction and subcutaneous implant locations.

groups, using a two-sided *t*-test in Microsoft Excel. Each group at each time point consisted of at least five data points. Data were used as collected, with no preprocessing. Significance was defined as $p \leq 0.05$.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgements

The authors thank Tony Rowe and Meg Evans at the Commonwealth Scientific and Industrial Research Organization (North Ryde, NSW, Australia) for their efforts during the in-life portion of the rabbit study. Thanks to Randy D. White at Preclintox Services, LLC (Hyrum, UT) and Mark Jordi at JordiLabs, LLC (Mansfield, MA) for their extensive work on the extractables and leachables toxicological assessment. Finally, the authors acknowledge both the American Preclinical Services (Minneapolis, MN) and WuXi AppTec (St. Paul, MN) for the completion of multiple biocompatibility testing protocols. This research was funded in its entirety by Foldax, Inc., Salt Lake City, UT, USA. The Conflict of Interest statement was corrected on February 08, 2021 after initial publication online.

Conflict of Interest

All authors are either consultants to or employees of Foldax, Inc.

Keywords

biocompatibility, biostability, polyurethane, synthetic valves, thrombogenicity, toxicological risk assessment

Received: September 11, 2020

Revised: October 29, 2020

Published online: December 21, 2020

- [1] C. Prisacariu, *Polyurethane Elastomers from Morphology to Mechanical Aspects*, Springer, New York 2011.
- [2] J. O. Akindoyo, M. D. H. Beg, S. Ghazali, M. R. Islam, N. Jeyaratnam, A. R. Yuvaraj, *RSC Adv.* **2016**, 6, 114453.
- [3] N. K. W. K. Lambla, S. L. Cooper, *Polyurethanes in Biomedical Applications*, CRC Press, New York 1998.
- [4] P. A. Gunatillake, L. S. Dandeniyage, R. Adhikari, M. Bown, R. Shanks, B. Adhikari, *Polym. Rev.* **2019**, 59, 391.
- [5] E. M. Christenson, J. M. Anderson, A. Hiltner, *Corros. Eng. Sci. Technol.* **2007**, 42, 312.
- [6] K. B. Stokes, *J. Biomater. Appl.* **1988**, 3, 228.
- [7] K. Stokes, P. Urbanski, J. Upton, *J. Biomater. Sci. Polym. Ed.* **1990**, 1, 207.
- [8] D. J. Martin, G. F. Meijs, G. M. Renwick, S. J. McCarthy, P. A. Gunatillake, *J. Appl. Polym. Sci.* **1996**, 62, 1377.
- [9] P. A. Gunatillake, G. F. Meijs, E. Rizzardo, R. C. Chatelier, S. J. McCarthy, A. Brandwood, K. Schindhelm, *J. Appl. Polym. Sci.* **1992**, 46, 319.
- [10] A. Brandwood, G. F. Meijs, P. A. Gunatillake, K. R. Noble, K. Schindhelm, E. Rizzardo, *J. Biomater. Sci. Polym. Ed.* **1994**, 6, 41.
- [11] P. A. Gunatillake, G. F. Meijs, S. J. McCarthy, R. Adhikari, N. Sherriff, *J. Appl. Polym. Sci.* **1998**, 69, 1621.
- [12] T. Choi, J. Weksler, A. Padsalgikar, J. Runt, *Polymer* **2010**, 51, 4375.
- [13] R. Adhikari, P. A. Gunatillake, S. J. McCarthy, C. F. Meijs, *J. Appl. Polym. Sci.* **2000**, 78, 1071.
- [14] T. Choi, J. Weksler, A. Padsalgikar, J. Runt, *Polymer* **2009**, 50, 2320.
- [15] T. Choi, J. Weksler, A. Padsalgikar, J. Hernandez, J. Runt, *Aust. J. Chem.* **2009**, 62, 794.
- [16] A. Simmons, J. Hyvarinen, R. A. Odell, D. J. Martin, P. A. Gunatillake, K. R. Noble, L. A. Poole-Warren, *Biomaterials* **2004**, 25, 4887–4900.
- [17] G. F. Meijs, P. A. Gunatillake, S. J. McCarthy, US Patent 6,313,254-B1, **2001**.
- [18] G. F. Meijs, E. Rizzardo, A. Brandwood, P. Gunatillak, K. H. Schindhelm, US Patent 5,393,858-A, **1995**.
- [19] P. A. Gunatillake, D. J. Martin, G. F. Meijs, S. J. McCarthy, R. Adhikari, *Aust. J. Chem.* **2003**, 56, 545.
- [20] D. J. Martin, L. A. P. Warren, P. A. Gunatillake, S. J. McCarthy, G. F. Meijs, K. Schindhelm, *Biomaterials* **2000**, 21, 1021.
- [21] R. Hernandez, J. Weksler, A. Padsalgikar, J. Runt, *J. Biomed. Mater. Res. Part B, Appl. Biomater.* **2008**, 87A, 546.
- [22] S. J. McCarthy, G. F. Meijs, N. Mitchell, P. A. Gunatillake, G. Heath, A. Brandwood, K. Schindhelm, *Biomaterials* **1997**, 18, 1387.
- [23] *Abbott Product Performance Report*, 1st ed., Abbott, Chicago, IL **2020**, p. 270, 318.
- [24] K. A. Chaffin, A. J. Buckalew, J. L. Schley, X. J. Chen, M. Jolly, J. A. Alkatout, J. P. Miller, D. F. Untereker, M. A. Hillmyer, F. S. Bates, *Macromolecules* **2012**, 45, 9110.
- [25] J. Kang, J. P. Kennedy, *J. Polym. Sci. Part A: Polym. Chem.* **2015**, 53, 1.
- [26] K. A. Chaffin, C. L. Wilson, A. K. Himes, J. Dawson, T. D. Haddad, A. J. Buckalew, J. P. Miller, D. F. Untereker, N. K. Simha, *Biomaterials* **2013**, 34, 8030.
- [27] K. A. Chaffin, X. J. Chen, L. McNamara, F. S. Bates, M. A. Hillmyer, *Macromolecules* **2014**, 47, 5220.
- [28] A. Padsalgikar, E. Cosgriff-Hernandez, G. Gallagher, T. Touchet, C. Iacob, L. Mellin, A. Norlin-Weissenrieder, J. Runt, *J. Biomed. Mater. Res. Part B, Appl. Biomater.* **2015**, 103, 159.
- [29] E. Cosgriff-Hernandez, E. Tkatchouk, T. Touchet, N. Sears, A. Kishan, C. Jenney, A. D. Padsalgikar, E. Chen, *J. Biomed. Mater. Res. Part B, Appl. Biomater.* **2016**, 104, 1805.
- [30] B. L. Wilkoff, J. Rickard, E. Tkatchouk, A. D. Padsalgikar, G. Gallagher, J. Runt, *J. Biomed. Mater. Res. Part B, Appl. Biomater.* **2016**, 104, 411.
- [31] D. Bezuidenhout, D. F. Williams, P. Zilla, *Biomaterials* **2015**, 36, 6.
- [32] L. S. Dandeniyage, R. Adhikari, M. Bown, R. Shanks, B. Adhikari, C. D. Easton, T. R. Gengenbach, D. Cookson, P. A. Gunatillake, *J. Biomed. Mater. Res. Part B, Appl. Biomater.* **2018**, 106, 1712.
- [33] L. S. Dandeniyage, R. Adhikari, M. Bown, R. Shanks, B. Adhikari, C. D. Easton, T. R. Gengenbach, D. Cookson, P. A. Gunatillake, *J. Biomed. Mater. Res. Part B, Appl. Biomater.* **2019**, 107, 112.
- [34] L. S. Dandeniyage, W. Knowler, R. Adhikari, M. Bown, R. Shanks, B. Adhikari, P. A. Gunatillake, *J. Biomed. Mater. Res. Part B, Appl. Biomater.* **2019**, 107, 2557.
- [35] L. S. Dandeniyage, R. Adhikari, M. Bown, R. Shanks, B. Adhikari, P. A. Gunatillake, *Mater. Today Comm.* **2019**, 18C, 110.
- [36] Foldax Inc., proprietary data not published.
- [37] A. N. Gent, P. B. Lindley, A. G. Thomas, *J. Appl. Polym. Sci.* **1964**, 8, 455.
- [38] L. A. Harker, S. R. Hanson, *J. Clin. Invest.* **1979**, 64, 559.
- [39] L. A. Harker, U. M. Marzec, A. B. Kelly, N. R. F. Chronos, I. B. Sundell, S. R. Hanson, J. M. Herbert, *Circulation* **1998**, 98, 2461.
- [40] G. Gallagher, A. Padsalgikar, E. Tkatchouk, C. Jenney, C. Iacob, J. Runt, *J. Biomed. Mater. Res. Part B, Appl. Biomater.* **2017**, 105, 1544–1558.
- [41] S. Gad-McDonald, S. C. Gad, in *Biomaterials, Medical Devices, and Combinations Products: Biocompatibility Testing and Safety Assessment* (Eds.: S. C. Gad, S. Gad-McDonald), CRC Press, Boca Raton, FL **2016**.
- [42] R. Ward, J. Anderson, R. McVenes, K. Stokes, *J. Biomed. Mater. Res.* **2006**, 77A, 580.
- [43] D. C. MacGregor, L. Pinchuk, M. C. Esquivel, J. B. Martin Jr., G. J. Wilson, *PACE* **1991**, 14, 694.
- [44] D. J. Martin, L. A. P. Warren, P. A. Gunatillake, S. J. McCarthy, G. F. Meijs, K. Schindhelm, *Biomaterials* **2001**, 22, 973.